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DTU Environment
Department of Environmental Engineering

Technical University of Denmark

Increasing the precision of microplate measurements of algal growth rate

Jonathan Van Wagenen*, Alejandro Moure Abelenda, Davide De Francisci, Susan Løvstad Holdt, Irini Angelidaki

*jovw@env.dtu.dk DTU Environment, Department of Environmental Engineering, Technical University of Denmark, Miljøvej, Building 113, 2800 Kgs. Lyngby, DENMARK

Introduction

The Technical University of Denmark will collaborate with Cluster Biofuels Denmark in the operation of a new pilot photobioreactor facility as a part of the E4WATER project. A critical challenge in the application of algal technologies is to move from small scale evaluation of many conditions to the pilot plant in a short period of time. For this reason, a microwell plate system was designed to screen different strains of algae on different industrial wastes. Preliminary results have shown it possible to increase the observed period of exponential growth by measuring the fluorescence of low-density cultures in microwell plates, thereby allowing better quantification of the exponential growth rate. How these rates of growth translate into larger scale cultures will soon be determined .

Aims

The number of conditions that can be measured needs to be increased in order to allow screening of large number of algal species. One critical aspect is to increase the observable length of exponential growth rate to a period of several days. This study aims to:

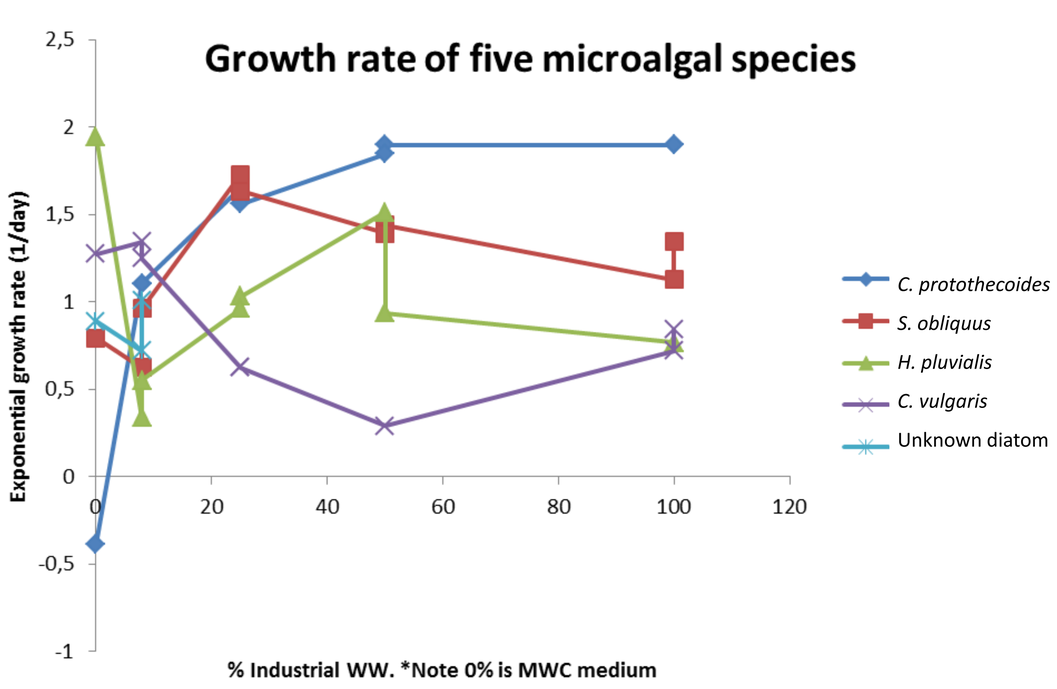
- Compare the sensitivity of the detection methods of the microplate reader (Synergy, BioTek) in respect to optical density and fluorescence
- Demonstrate that more sensitive measurements enable better quantification of the maximum specific growth rate.
- Apply the improved protocol to the screening of algal growth on industrial wastewater.

Results: Limits of Detection and Quantification

	Optical Density		Florescence		Ratio
Species	CL	QL	CL	QL	(OD : florescence)
S. obliquus	6,7E+04	2,2E+05	5,5E+03	1,8E+04	**12**
C. vulgaris	2,3E+05	7,7E+05	4,7E+03	1,6E+04	**50**
H. pluvialis	3,6E+04	1,2E+05	2,0E+03	6,7E+03	**18**
C. protothecoides	1,2E+05	3,9E+05	3,3E+03	1,1E+04	**36**
Average	1,1E+05	3,8E+05	3,8E+03	1,3E+04	**29**

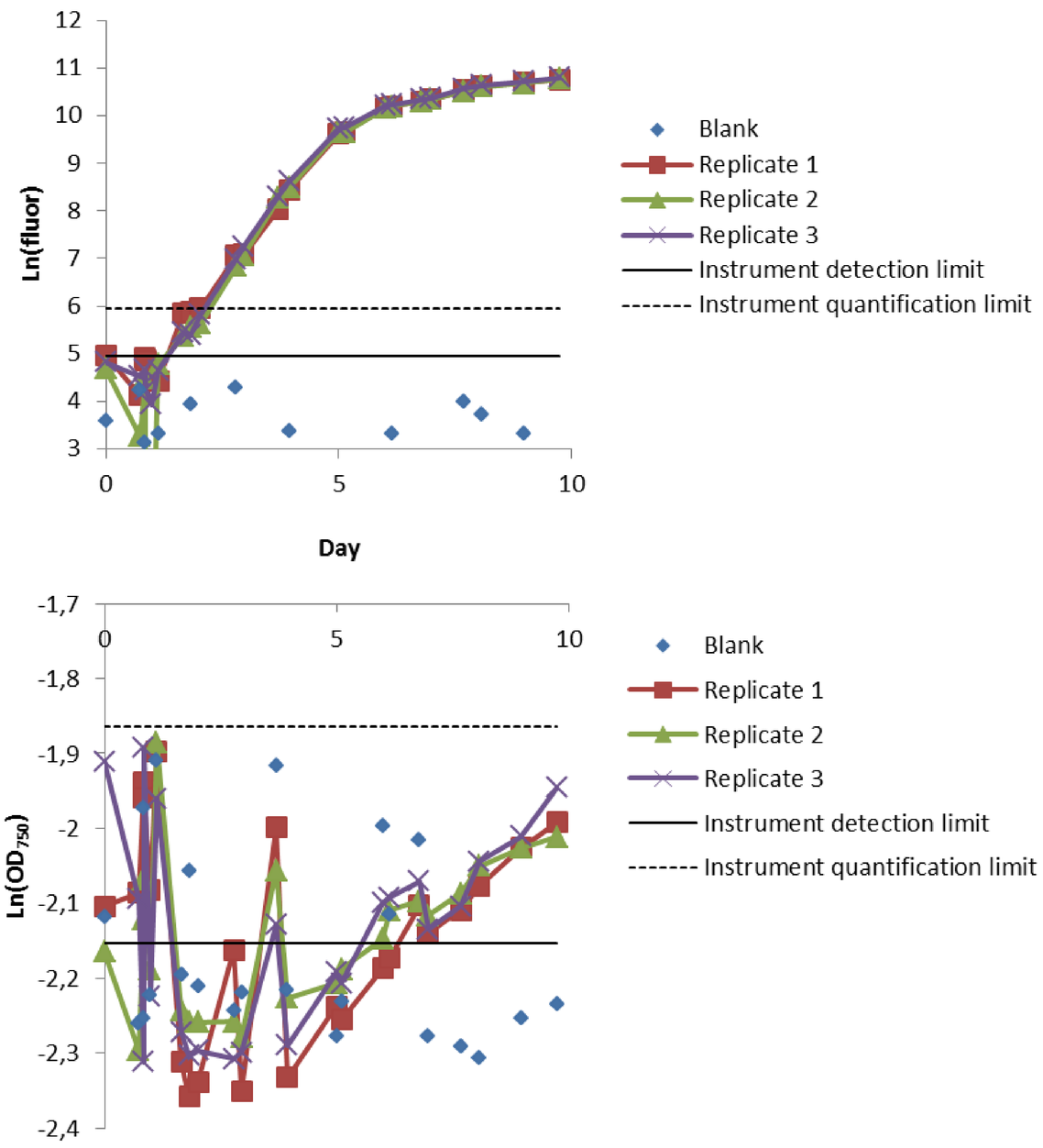
Determination of limit of detection (CL) and limit of quantification (QL). (Units = cell/mL). The limits of detection and limits of quantification were determined for each species of microalgae using the IUPAC protocol. Optical density was determined to be 12 to 50 fold less sensitive than florescence.

Application: Screening of Industrial Wastewater



Results of screening microalgae on industrial (pharmaceutical) wastewater (WW). This diluted WW was tested as growth medium for different species of microalgae: *Chlorella protothecoides, Scenedesmus obliquus, Haematoccocus pluvialis, C. vulgaris* and an unknown isolated diatom from the industrial waste water

Importance of Instrument Sensitivity



Growth curve of *Chlorella vulgaris*. Fluorescence and optical density signals were logarithmically transformed in order to calculate the exponential specific growth rate. A period of exponential increase between day 2 and 5 was observable by using fluorescence (excitation 440, emission 690 nm)(top Figure). The optical density (750 nm) signal did not exceed the detection limit until day 7, by which time declining growth was seen in the fluorescence signal (bottom Figure).

Conclusions

These experiments show that the period where exponential growth rate is measurable can be increased by selecting a more sensitive method of detection. Furthermore, it was seen that:

- Fluorescence was 12 to 50 times more sensitive than optical density
- The period of exponential growth was more easily observable with fluorescence.
- In the tested wastewater, *Chlorella protothecoides* was the fastest growing species.
- Other screened species also grew faster in WW than on the standard MWC medium.

This technique is applicable in any context that deals with screening multiple strains of microalgae or multiple media compositions. However, before these results can be generalized it is important to develop an understanding of the best way to translate results obtained in microplates to industrially relevant scales.

Acknowledgement and References

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